

Attorney Docket No. 2002_0426A
Serial No. 10/089,040
June 8, 2006

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A set of probes for analyzing protein A - protein B interaction, which comprises:

probe "a" comprising an ~~N-half~~ N-terminal portion of an intein polypeptide and an ~~N-half~~ N-terminal portion of an indicator protein, wherein the ~~N-half~~ N-terminal portion of the indicator protein is ~~connected at~~ fused to the ~~N-terminal end~~ N-terminus of the ~~N-half~~ N-terminal portion of the intein polypeptide, and the ~~C-terminal end of the N-half~~ C-terminus of the intein polypeptide is ~~a site for connecting~~ capable of fusing with a target protein A; and

probe "b" comprising a ~~C-half~~ C-terminal portion of the intein polypeptide and a ~~C-half~~ C-terminal portion of the indicator protein, wherein the ~~C-half~~ C-terminal portion of the indicator protein is ~~connected at~~ fused to the ~~C-terminal end~~ C-terminus of the ~~C-half~~ C-terminal portion of the intein polypeptide, and the ~~N-terminal end of the C-half~~ N-terminus of the intein polypeptide is ~~a site for connecting~~ capable of fusing with a target protein B.

wherein the indicator protein is a green fluorescent protein or a luminescent enzyme.

2. (Cancelled)

3. (Previously presented) The set of probes for analyzing protein A - protein B interaction analysis of claim 1, wherein the C-terminal of probe "a" and the N-terminal of probe "b" each contain a linker sequence.

4-8. (Cancelled)

9. (Currently amended) The set of probes for analyzing protein A - protein B interaction of claim 8 1, wherein the luminescent enzyme is a luciferase.

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10. (Currently amended) A method for analyzing protein A - protein B interaction by using the set of probes of claim 1, which comprises:

~~connecting protein A with probe "a", and connecting protein B with probe "b" fusing the C-terminus of the intein polypeptide of probe "a" with target protein A, and fusing the N-terminus of the intein polypeptide of probe "b" with target protein B;~~

~~introducing probe "a" and probe "b" in a system under conditions permitting excision of the intein portions of probes "a" and "b" upon interaction of proteins A and B; and~~

~~detecting the interaction of protein A with protein B by measuring a change of a signal from the indicator protein that is a fusion protein consisting of the ~~N-terminal-half~~ N-terminus of the indicator protein and the ~~C-terminal-half~~ C-terminus of the indicator protein.~~

11. (Cancelled)

12. (Currently amended) A vector expressing a set of probes for analyzing protein A - protein B interaction, which co-expresses probe "a" comprising a fusion polypeptide of an ~~N-half~~ N-terminal portion of an intein polypeptide and an ~~N-half~~ N-terminal portion of an indicator protein, and probe "b" comprising a fusion polypeptide of a ~~C-half~~ C-terminal portion of the intein polypeptide and a ~~C-half~~ C-terminal portion of the indicator protein, wherein the vector comprises:

~~a polynucleotide encoding the fusion polypeptide of probe "a", wherein the coding region for the ~~N-half~~ N-terminal portion of the indicator protein is ligated at 5' side of the coding region for the ~~N-half~~ N-terminal portion of the intein polypeptide, and a 3' side of the coding region for the ~~N-half~~ N-terminal portion of the intein polypeptide is a cloning site for ligating the polynucleotide encoding protein A; and~~

~~a polynucleotide encoding the fusion polypeptide of probe "b", wherein the coding region for the ~~C-half~~ C-terminal portion of the indicator protein is ligated at 3' side of the coding region for the ~~C-half~~ C-terminal portion of the intein polypeptide, and a 5' side of the coding region for the ~~C-half~~ C-terminal portion of the intein polypeptide,~~

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C-terminal portion of the intein polypeptide is a cloning site for ligating the polynucleotide encoding protein B,

wherein the polynucleotide encoding the indicator protein encodes a green fluorescent protein or a luminescent enzyme.

13. (Previously presented) A method for analyzing protein A - protein B interaction by using the expression vector of claim 12, which comprises:

ligating the polynucleotide encoding protein A into the expression vector to the 3' side of the coding region for the N-terminal portion of the intein polypeptide and ligating the polynucleotide encoding protein B into the expression vector to the 5' side of the coding region for the C-terminal portion of the intein polypeptide;

introducing the vector into a eukaryotic cell and thereby expressing probe "a" connecting fusing the C-terminus of the intein polypeptide to protein A in the eukaryotic cell and expressing probe "b" connecting fusing the N-terminus of the intein polypeptide to protein B in the eukaryotic cell, respectively, under conditions permitting excision of the intein portions of probes "a" and "b" upon interaction of proteins A and B; and

detecting the interaction of protein A with protein B by measuring a change of a signal from the indicator protein that is a fusion protein of the ~~N-terminal half~~ N-terminus of the indicator protein and the ~~C-terminal half~~ C-terminus of the indicator protein.